

“overexpressed”. Examiner Lubet indicated that this amendment would be sufficient to overcome the rejection.

2. Claims 1-7 are enabled under 35 USC §112, first paragraph.

Claim 1 has been amended as discussed in the interview. Applicants submit that in view of the amendments to the claims, this rejection of Claims 1-7 as lacking enablement should be withdrawn.

As discussed in the interview, the Office Action raised three particular issues regarding the enablement of the claims: (1) the method of administration of the heparinase enzyme, (2) the number of the inflammatory diseases within the scope of the claims, and (3) the number of heparinase enzymes falling within the scope of the claims. Each of these issues was discussed in the interview and is further discussed below.

In response to the first issue, Applicant’s have amended the claims to recite “intravascular administration”, support for which amendment can be found at least in Examples 7 and 8, in particular see page 35, lines 25-32, page 37, lines 32-35, and page 41, lines 26-30.

In response to the second issue, Applicants have amended the claims to a method to decrease localized inflammatory responses arising from an ischemia/reperfusion injury in a tissue of a patient, support for which amendment can be found at least in Examples 7 and 8, see pages 35-49 (specifically page 39, line 15 through page 40, line 20), which Examples demonstrated in two *in vivo* animal model systems that administration of heparinase enzyme decreases localized inflammatory response arising from an ischemia/reperfusion injury.

In response to the third issue, Applicants have amended the claims to recite the functional properties of the heparinase enzyme to be administered, thus specifically defining which heparinase enzymes will be useful in the claimed invention. The claims as amended recite that heparinase enzyme must “decrease neutrophil transmigration through activated endothelium and basement membrane of said vasculature which decreases said localized inflammatory response arising from an ischemia/reperfusion injury”. This amendment specifies the necessary *in vivo* enzymatic action of the heparinase enzyme that is required in order to achieve the claimed effect. One of ordinary skill in the art could readily determine whether or not a specific heparinase enzyme decreased neutrophil transmigration through the endothelium and basement membrane

by following the protocol described in Example 5 (pages 31-33) which protocol is an accepted model system commonly used to analyze conditions affecting neutrophil transmigration across activated endothelium (see page 31, lines 13-26). The protocol described in Example 5 was utilized to determine that each of heparinase I, heparinase II, heparinase III (see Example 5) and heparan sulfate degrading substance found in human platelets β -thromboglobulin (see Example 6, see page 34, lines 5-19 and page 35, lines 13-19) are useful to decrease neutrophil transmigration through activated endothelium and basement membrane of the vasculature. This *in vitro* assay to measure neutrophil transmigration across activated endothelium and basement membrane of the vasculature is a modification of model systems for assessment of neutrophil transmigration to inflamed subendothelial tissues, (see Cotran et al., page 47 (cited by the Examiner), and Huber et al., 1991, Science, 254:99-102, see the legend for Figure 1 which provides the description of the model system (copy attached)).

The Examiner requested in the interview that the link between a decrease in neutrophil transmigration across the endothelium and basement membrane of the vasculature and a decrease in the localized inflammatory response be further explained. As stated at the interview, localized inflammation has long been established to result from leukocyte transmigration across the vascular endothelium and basement membrane to the site of tissue injury, and that the first of such leukocytes to transmigrate are neutrophils, (see, for example, Cotran et al. cited by the Examiner, Huber et al., cited above, and Lefer and Lefer, 1993, Ann. Rev. Pharmacol. Toxicol, 33:71-90, (copy attached)).

As described in Cotran et al., cited by the Examiner, the accumulation of leukocytes is the most important feature of the inflammatory reaction, and in most types of acute inflammation, neutrophils predominate first (see page 46, second column, final paragraph). The sequence of leukocyte events during inflammation are stated on page 45 of Cotran et al. to be divided into (1) margination, (2) adhesion, (3) emigration toward a chemotactic stimulus ("transmigration"), (4) phagocytosis and intracellular degradation, and (5) extracellular release of leukocyte products. As stated by Cotran et al. on pages 46-47, leukocyte adhesion and subsequent emigration are key steps in inflammation, and the presence of cytokines is required for such adhesion and transmigration by neutrophils. Disruption of these steps serves to decrease the localized inflammatory response.

As described in Huber et al., binding of neutrophils to inflamed endothelium initiates an orchestrated series of events in which neutrophils bind to the surface of the endothelium and then penetrate the vessel wall and proceed into the interstitium (see page 99, 1st paragraph). After presenting evidence that heparin bound chemotactic factors induce neutrophil adherence and transmigration in the model system, Huber et al., concludes by noting that the development of inhibitors of the chemotactic factors could lead to the development of anti-inflammatory therapies (see page 101, third column, final paragraph). As detailed in the specification, Applicants have identified such an inhibitor of inflammation.

As described in Lefer et al., ischemia leads to hypoxia which, if severe enough, can lead to reduced energy metabolism and then to a slow but significant degree of tissue injury and necrosis, which tissue injury is further enhanced and accelerated by reperfusion (see page 75, first paragraph). Reperfusion, through a series of alterations of the vascular endothelium, leads to polymorphonuclear ("PMN") leukocyte (i.e. neutrophil) adherence which leukocytes transmigrate through the vascular endothelium (by diapedesis) and localize to compromised cells where they release a host of pro-inflammatory mediators which in turn promote cell injury (see pages 75-76, bridging paragraph). Lefer states that a agent which can inhibit neutrophils or their mediators would preserve endothelial function and thus reduce inflammation that would otherwise arise from ischemia/reperfusion (see, page 76, final paragraph and pages 80-81 (specifically page 81, second paragraph).

Applicants demonstrated in Example 7, pages 35-39 of the specification, that intravascular administration of heparinase enzyme will decrease neutrophil adhesion and transmigration through activated endothelium and basement membrane of the vasculature subsequent to an ischemic event thereby decreasing the localized inflammatory response which would have otherwise arisen from an ischemia/reperfusion injury to tissue. Specifically, in this example intravital video microscopy was used to quantitate neutrophil movement through the vasculature after an ischemic event in rats, and neutrophil movement in untreated rats was compared with that in heparinase treated rats. As described in the specification, pages 38, line 28 through 39, line 8 and shown in Figures 12-14, in untreated rats the number of leukocytes which adhered to the endothelial walls of the vasculature and extravasated progressively increased during reperfusion, but in heparinase treated rats no significant difference was

observed for either leukocyte adhesion or extravasation when compared to control animals which had not been subject to an ischemic event.

With regard to new Claim 18, the Examiner has previously indicated that such a claim would be allowable.

With regard to new claim 19, the Examiner requested a showing that shock is considered an ischemia/reperfusion injury. Applicants refer the Examiner to Lefer et al., pages 82-83, which teaches "Finally, endothelial dysfunction can be studied in whole body ischemia-reperfusion (i.e. circulatory shock states). These whole body ischemia-reperfusion states in which endothelial dysfunction has been observed include endotoxic shock, traumatic shock and hemorrhagic shock" (see specifically, page 82-83 bridging sentences). Thus, shock, as recited in new claim 19 is an ischemia/reperfusion injury which gives rise to an inflammatory response that can be decreased by the administration of heparinase.

Based on the discussion at the interview and foregoing remarks, the Examiner is respectfully requested to reconsider and withdraw the enablement rejection of claims 1-7.

3. The claims are novel under 35 USC § 102(e) and (f) in view of the cited art.

Claims 1-7 were rejected under 35 USC § 102(e) or (f) over Zimmermann US 5,997,863. The Examiner states that Zimmermann teaches a method of treating ischemia in a rabbit hind limb ischemic model by administering heparinase I (see Example 8, columns 17-18). This rejection is respectfully traversed.

As discussed in the interview, Example 8 of Zimmermann et al. does not teach a method of decreasing localized inflammatory responses arising from an ischemia/reperfusion injury in a tissue, but rather teaches that heparinase I is useful to promote revascularization (also known as angiogenesis) of tissue that has been subjected to ischemia when administered for several days beginning 10 days after the ischemic event. At such time any localized inflammatory response which would have arisen from an ischemia/reperfusion injury has already occurred, and the tissue is in the process of healing. As discussed in the interview, wound healing is process that is distinct from inflammation. Zimmermann describes that the wound healing process is generally divided into three temporally overlapping phases, inflammation, proliferation and remodeling (col. 2, lines 60-62). Thus, as stated in Zimmermann, inflammation is considered a facet of

wound healing, rather than the other way around as suggested by the Examiner. Further, Zimmermann teaches that during the inflammation stage, blood borne cells infiltrate the wound site (col. 2, lines 63-64) and that angiogenesis occurs during the proliferative phase of wound healing (col. 3, lines 13-18). Thus, as stated above, Example 8 of Zimmermann does not anticipate the claimed invention.

4. The claims are patentable under 35 USC § 103 in view of the cited art.

Claims 1-7 remain rejected under 35 USC §103(a) as being unpatentable in view of a combination of ten references. Specifically, Hoogewerf et al, Gilat et al. (X), Vlodavsky et al., Zimmermann US Patent 5,169,722, Fuks et al. US Patent 5,362,641 and Sasisekharan et al. US Patent 5,567,417 in view of the teachings of Nash et al., Lider et al., Ratner et al. and Gilat et al. (AA) were stated to make the invention of using heparinase enzyme to decrease a localized inflammatory response. This rejection is respectfully traversed.

As noted by the Examiner, each of Hoogewerf et al, Gilat et al. (X), Vlodavsky et al. and Zimmermann US Patent 5,169,722 teach heparinase enzymes obtained from different sources but none of these articles teach the use of these heparinase enzymes to decrease an inflammatory response, nor that a heparinase enzyme decreases neutrophil transmigration through activated vascular endothelium and basement membrane.

Each of Fuks et al. US Patent 5,362,641 and Sasisekharan US Patent 5,567,417 was cited for their teachings that heparinases are useful to “treat localized inflammatory responses in a variety of diseases” (Office Action, page 9). Review of Fuks et al. reveals that a human heparanase is taught to be useful to promote wound healing, specifically by promotion of angiogenesis. See, Fuks et al., col. 4, line 52 through col. 5, line 6. Review of Sasisekharan et al. reveals that bacterial heparinases I and III are taught to be useful to inhibit neovascularization. As discussed above in Section 3, neovascularization is one step of wound healing, but wound healing is not a facet of inflammation, rather inflammation is generally considered to be an initial, but not absolutely necessary, step of the wound healing process. Applicant respectfully submits that neither of Fuks et al. or Sasisekharan et al. teaches the use of a heparinase enzyme to decrease neutrophil transmigration through activated endothelium and basement membrane of

the vasculature thus decreasing a localized inflammatory response arising from an ischemia/reperfusion injury.

The Examiner's specific application of the primary references to each of the secondary references is not detailed in the Office Action. On pages 9-10 of the Office Action, the Examiner generally states that it would have been obvious to locally administer heparinase enzymes as taught in the primary references with the expectation that inflammatory responses would be decreases as taught in Fuks et al., Sasisekharan et al., Nash et al. and Lider et al. However, the teachings of Fuks et al., Sasisekharan et al. and Nash et al. regard angiogenesis, and as detailed above, angiogenesis is not a facet of inflammation. Further, Lider et al. teaches that heparinase can decrease the T cell mediated inflammatory response of delayed type hypersensitivity, which is distinct from an ischemia/reperfusion injury. Therefore the combination of either of Lider et al. or Nash et al. with the primary references does not teach or suggest the invention as recited in the amended claims.

No specific application of the primary references to the teachings of Ratner et al. and Gilat et al. (AA) were provided by the Examiner, however, neither of these secondary references teach or suggest that intravascular administration of heparinase enzyme will decrease neutrophil transmigration across activated endothelium and basement membrane of the vasculature which decreases the localized inflammatory response arising from an ischemia/reperfusion injury. Therefore the combination of either of Ratner et al. or Gilat et al. (AA) with the primary references does not teach or suggest the invention as recited in the amended claims.

Based on the foregoing remarks and the discussion in the interview, the Examiner is respectfully requested to reconsider and withdraw this rejection of claims 1-7.

5. Claims 1-7 are patentably distinct from US Patent 5,997,863.

The claims of US Patent 5,997,863 are drawn to methods of enhancing normal wound healing by administration of a heparinase enzyme from *Flavobacterium* and were asserted to be patentably indistinct from claims 1-7 of the instant application. This rejection is respectfully traversed.

As stated in the interview, US Patent 5,997,863 and the instant invention were commonly owned at the time the instant invention was made. However, as also discussed in the interview

and as presented in detail in Section 2 above, wound healing is a multifaceted process which may involve as an early step an inflammatory response, therefore, Claims 1-7 as amended are patentably distinct from the claims of US Patent 5,997,863. In view of the evidence presented in this response, Applicants respectfully request reconsideration and withdrawal of this rejection.

Summary

Entry of the present amendments and reconsideration of the amended application, in view of the interview conducted on May 9, 2000 and the foregoing remarks, are respectfully requested. The amended application should be in condition for allowance, and such action is respectfully requested. If the Examiner believes that a telephone conversation would expedite prosecution in this Application, the Examiner is invited to telephone the undersigned at (617) 526-6460.

Respectfully submitted,
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